special attention. These special investigations were undertaken by Mr. Chamberlain and Mr. Schaffner, who have made an independent presentation of their results, for which they are entirely responsible. This contribution, therefore, is made up of three distinct and independent papers, each with its own plates, but naturally brought together by the nature of the subject.

My own part is the organization of observations made by the group of students referred to, in so far as they pertain to the embryo sac, fertilization, and the embryo. Mr. Chamberlain, from his own observations, deals with the pollen grain; while Mr. Schaffner presents his own observations and conclusions in reference to certain cytological phenomena connected with the "reduction division" in the embryo sac.

The material used was fixed in Flemming's weaker solution, Merkel's fluid, 1 per cent. chromic acid, 1 per cent. chromic acid with a trace of acetic acid, and picric acid.

Xylol was used almost exclusively to precede the paraffine bath. Serial sections were cut with a Thoma microtome, usually 5 or 10 μ thick, and occasionally but 1 μ thick.

A large number of stains and combinations was used. Cyanin and erythrosin proved excellent for most stages in the development of the macrospore; Delafield's haematoxylin is to be recommended for embryos; safranin with gentian violet and orange G gave good results in staining pollen grains; Heidenhain's iron alum haematoxylin used alone or with erythrosin or orange G gave by far the best preparations for cytological study.

I

THE EMBRYO SAC AND ASSOCIATED STRUCTURES.

JOHN M. COULTER.

(WITH PLATES XXXII–XXXIV)

The results here recorded traverse ground which has become very familiar. It will not be necessary, therefore, to make extended mention of all the phenomena, but to discuss only

certain points which seem to merit comment. It seems best, however, to preserve the sequence of events for the benefit of those who may not have access to the more extensive papers. The students whose observations have supplied the data for this portion of the contribution, and whose individual contributions may be recognized by the initials appended to the different figures, are Otis W. Caldwell, John G. Coulter, Henry C. Cowles, T. C. Frye, Nina D. Holton, Florence M. Lyons, William D. Merrell, Mabel C. Merriman, and Wilson R. Smith.

DEVELOPMENT OF THE EMBRYO SAC.

A single large hypodermal archesporial cell very early makes its appearance, distinguished by its size, contents, and very prominent nucleus (*fig.* 1). No evidence of the cutting off of a tapetal cell, or a division into potential macrospores was detected. The sequence of cell divisions usual in angiosperms is entirely suppressed, and the archesporial cell develops directly into the macrospore (embryo sac). It will be remembered that there are three possibilities in what may arise from the archesporial cell of angiosperms. It may, and apparently usually does, give rise at its first division to a primary tapetal cell and a primary sporogenous cell, each of which may give rise to a more or less extensive cell progeny; or it may, less frequently, give rise to no tapetal region, but play the part of a primary sporogenous cell and divide into potential macrospores; or it may, apparently exceptionally, develop directly into the fertile macrospore. This extreme shortening of the history of the embryo sac, recorded as yet only for Lilium and certain allied liliaceous genera, obliterates the distinctions between archesporial cell, primary sporogenous cell or mother cell, and macrospore, so far as distinct cell existence is concerned, but what the ellipsis involves in nuclear and cytoplasmic changes is worthy of research. Certain it is, that this remarkable cell has a relatively long existence in the uninucleate condition, brought to a close by its rapid enlargement. As there is no tapetum, and no periclinal divisions occur in the epidermis to increase the mass of the nucellus toward the

micropyle, the encroachment of the enlarging macrospore is at first chiefly upon the tissues beneath.

This enlargement is the first step in the "germination of the macrospore," and when the sac has become considerably elongated the first nuclear division occurs near the center, the axis of the spindle being longitudinal (*fig. 2*), and the daughter nuclei passing to their polar positions. A more detailed view of the antipodal end of this first spindle, at an earlier stage, while the chromosomes are still distinct, is given in *fig. 3*, showing the usual transient cytoplasmic radiations about the chromosome group, and the definite relation of the larger ones to the micronucleoli. The cytological phenomena connected with this division, known as the "reduction division," form the subject of Mr. Schaffner's paper. With such an abbreviated history as that of the macrospore of Lilium the division representing the reduction division is evident, but in most angiosperms the place of this special division in the history of the macrospore is not so clear.

Immediately after the placing of the two nuclei the second divisions occur (*figs. 4–8*), the micropylar spindle being transverse, the antipodal one longitudinal. In *figs. 4* and *5* the reduction number of chromosomes is apparent in the micropylar spindle, while a largely increased chromatin mass is apparent in the antipodal spindle. Very soon the resulting nuclei shift their positions more or less (*figs. 7, 7a, 8*), so that the directions of the two spindles are lost. The persistence of the spindle fibers (*figs. 7, 7a*) is a common phenomenon in the embryo sac divisions, and often helps to indicate the shifting of the freed nuclei.

In this second division certain phenomena were noted by Miss Merriman which deserve mention. The occasional occurrence of multipolar spindles in Lilium is well known, and *figs. 9–12* may be taken to represent them. As these spindles are associated with exceptional conditions of the chromatin band, and occurred in a single ovary, they suggest a very unusual and possibly a pathological condition. With the claims made for the relation between the multipolar spindle and the bipolar

spindle, it is interesting to note that among the hundreds of embryo sac spindles of Lilium that passed under our observation, multipolar spindles were found in but a single ovary. In *fig. 9*, representing an antipodal spindle, the chromatin band seems to be arranged in continuous loops the full length of the spindle. In *fig. 10*, representing the micropylar spindle of the same sac, two segments of the chromatin band are arranged also in continuous loops. *Fig. 11*, from another sac of the same ovary, represents a strongly multipolar antipodal spindle, but with the chromatin band broken up into chromosomes; while *fig. 12*, the micropylar spindle from the same sac, shows a continuous looping of the chromatin band. The significance of these phenomena seems quite obscure, and their normal or abnormal character in the case of Lilium can only be ascertained by further investigation. If they represent a normal phase in the development of the bipolar spindle, their rarity would indicate either that it is a peculiarly ephemeral phase, or that it is not easily recognized. If they represent another method of spindle formation their exceptional occurrence might be easily accounted for; and the same may be said of the hypothesis that they represent spindles disorganized by sectioning or reagents. In these same figures (*figs. 9–12*) it will be noticed that the reagents used have brought out abundant striations in the cytoplasm, whose normal or abnormal character may be in question.

Various phases in the eight-nucleated stage of the embryo sac are represented by *figs. 13–16*. The varying directions of the spindles are evident, but in general the synergid spindle is transverse, and the spindles which give rise to the polar nuclei are longitudinal. It is plain that the synergids are sister nuclei, as are also the oosphere and the micropylar polar nucleus. It is also evident from the figures that if direct division occurs among the antipodal nuclei of Lilium our preparations give no evidence of it. An examination of Miss Sargent's figures,[2] which are cited as representing cases of direct division in the

[2] Annals of Botany, 10: 445–447. 1896.

antipodal region of *L. Martagon*, shows that they might be taken for cases of mitosis.

In connection with the spindles of the embryo sac attention should be called to the fact that the spindle fibers thicken in the equatorial plane as if preparatory to the formation of a cell wall (*figs. 14, 15*). This phenomenon has been taken to be another evidence of the descent of this free-celled gametophyte from one of compact tissue.

The condition of things represented by *fig. 17* is difficult to interpret. While it is no unusual thing for a partition wall to be formed at the antipodal end of the sac, a wall at the micropylar end seems worthy of comment. If the nuclear division represented as just taking place is the first division of the definitive nucleus, which seems probable, the other nuclei are easily referred, and it would follow that the synergids are cut off from the oosphere (or oospore?) by a wall.

In our preparations, the fusion of the polar nuclei is so commonly associated with the fusion of the sex cells (*fig. 19*) that the so-called "eight-celled" stage of the sac may be regarded as its ordinary ante-fertilization preparation. *Figs. 18* and *19* represent the fusion of polar nuclei, in the latter case but a small portion of the upper nucleus being shown.

PHENOMENA OF FERTILIZATION.

The pollen tube, as usual, passes between a synergid and the wall of the sac, and then bends more or less sharply towards the oosphere. Its enlarged caliber and more deeply staining contents are associated with the disorganization of the synergid with which it is in contact. If the pollen tube has been directed to the micropyle under the influence of chemotaxis, and the active principle of chemotaxis is a secretion from the synergids, it is interesting to observe that when the tube has reached and passed the synergids it is under the control of an influence powerful enough to bend it sharply towards the oosphere. If the hypothesis of chemotaxis and the origin of the attractive substance are true in this case, it would seem that it does not effect

the essential contact, but brings the tube within the influence of another attraction which immediately directs it to the oosphere. The discharge of a male cell seems to be attended by disorganization and rupture of the tip of the tube (*figs. 19, 20, 24*), as observed by Schaffner in *Sagittaria variabilis*.[3] In *fig. 19* the second undischarged male cell may be seen in the end of the tube in a disintegrating condition; and *fig. 24* represents a case of a remarkably persistent tube and an undischarged male nucleus, the latter being distinctly nucleolated, as late as the second division of the embryo. The synergid not disorganized by the pollen tube persists for some time, as is usual, its nucleus being shown in *figs. 20, 22, 23*.

During fusion the sex nuclei hold no definite position in reference to each other, as is evident from *figs. 19–22*, where certain details concerning nucleoli and chromatin bands may also be noted. It is evident, thererefore, that the position of the fusing nuclei holds no relation to the plane of the first division of the oospore.

In *figs. 20* and *21* the structures figured by Guignard as centrospheres are represented. In these special cases no other structures in the cell bore any resemblance to them, but they were not seen except occasionally in connection with the nuclei in an advanced state of fusion. As no effort was made to demonstrate them, however, this testimony has no special significance. Their frequent association with nuclear phenomena in the higher plants certainly requires explanation, whether the current homology and function be established or not.

DEVELOPMENT OF THE EMBRYO.

Before division the oospore enlarges, elongates, and is not always axially attached to the sac wall (*fig. 23*). At the same time the nucleus enlarges and establishes itself at the free end of the cell. As a consequence, the first division, which is always transverse, results in a small apical cell and a comparatively large and somewhat vesicular basal cell. This basal cell and the

[3] BOT. GAZ. 23: 256. 1897.

subsequent basal region is worthy of remark, and will be noted later. After the first division there is no regular sequence of cell divisions. The second may occur in the basal cell, either transversely (*fig. 25*) or longitudinally (*fig. 24*). That a longitudinal division of the basal cell commonly occurs at some time is evidenced by the later stages of the embryo (*figs. 27–33*). Cell division continues in any region of the embryo and in every direction (*figs. 26–33*). It is impossible to formulate even a general sequence of events or to make any sharp distinction between suspensor and embryo. The amount of the whole embryonic structure which contributes to the completed embryo is variable, and such a thing as a distinctly defined suspensor which may "contribute" to the formation of the embryo does not exist. It would seem better to regard the so-called suspensor not as an organ distinct from the embryo, but rather as a region of the embryo, more or less extensive even in the same species, set apart to serve a temporary purpose. From this standpoint the question as to what the suspensor "contributes" to the embryo, and what the embryo "contributes" to the suspensor, becomes arbitrary and useless refinement. Like much physiological differentiation this may result in a structure externally distinct or it may not. The function of this region of the embryo seems to be to anchor, to absorb, and to relate the embryo properly to its food supply. Therefore, it displays the widest possible variation in extent and structure. The statement that certain plants have no suspensors may or may not be true, but this fact would seem to have no special morphological significance. It has seemed best to me to regard the suspensor not as a phylogenetic rudiment, but as a specialized structure of the embryo adapted to the peculiar conditions of intraseminal development.

The tendency of the basal region of the embryo to spread widely as an absorbent organ in contact with the wall of the sac is very noticeable (*figs. 26–32*). An extreme case at an early stage is represented by *fig. 29*, but cases of still more extensive lateral extension were observed, very suggestive of certain

reported cases of apogamous polyembryony from the nucellar tissue just above the embryo sac. In *fig. 32*, the most mature embryo represented, a distinct development of tissue in the suspensor region is shown, which appears late in the development of the embryo, and is well marked off from the embryo proper by a narrow neck. This suspensor tissue is erythrophilous as compared with the embryo, showing its closer relation to nutritive supplies. From this late development of tissue in the suspensor region, and its great activity, it would suggest its possible association with supposed cases of polyembryony.

DEVELOPMENT OF ENDOSPERM.

It is becoming well known that the first division of the definitive nucleus holds no direct relation to the fusion of the sexual nuclei. It may precede or follow this fusion, or be coincident with it. So far as observed it occurs after the entrance of the pollen tube into the sac, but at almost any time thereafter, apparently related to no special one of those events which follow and which go to make up the process of fertilization. It seems reasonable to suppose that the inciting event is the entrance of the tube. In the case of Lilium the sexual and polar pairs of nuclei were observed to fuse simultaneously, but when division begins the endosperm nuclei divide more rapidly than do the cells of the embryo. When the embryo is but two or three-celled numerous free endosperm nuclei are scattered throughout the embryo sac (*fig. 35*). Later cell walls begin to form in the endosperm. A very interesting phase in the division of the definitive nucleus is shown in *fig. 34*. The remarkably distinct radiations about the forming daughter nuclei seem to be due to the spindle fibers pulled apart, and to other radiations which are similar to those which appear about the forming daughter nuclei in the divisions of the nuclei of the embryo sac which precede fertilization. Such radiations are difficult to interpret, but their distinctness in this preparation could not be exaggerated.

During the development of the embryo and endosperm the

embryo sac enlarges rapidly except at the antipodal end, which is left as a sort of caecum, often thrust to one side, in which may be seen the disintegrating antipodal nuclei (*fig. 35*). Around the narrowed antipodal end of the sac there is developed a very heavy wall, which in itself would seem to be a sufficient reason for the failure of that region of the sac to enlarge.

EXPLANATION OF PLATES XXXII–XXXIV.

The figures are reduced from drawings to about three-eighths of their original size. The combination of objective and ocular is indicated in each case, the initial letters indicating Zeiss, Leitz, Reichert, and Bausch and Lomb. The four combinations used and their magnification in diameters were R$\frac{1}{7}$ R4, 760; B & L$\frac{1}{12}$ immersion R4, 1200; Z$\frac{1}{12}$ immersion L4; Z$\frac{1}{12}$ immersion Z18, 2250. All the figures are from *Lilium Philadelphicum* unless otherwise indicated.

FIG. 1. Tip of nucellus with the single archesporial cell which develops directly into the macrospore. B & L$\frac{1}{12}$ R4.

FIG. 2. First division of the macrospore nucleus, showing radiations about the daughter nuclei, and thickening of spindle fibers in the equatorial region. Z$\frac{1}{12}$ L4.

FIG. 3. Daughter nucleus of the first division at an earlier stage, showing relation of micronucleoli to radiations. Z$\frac{1}{12}$ Z18. Iron alum.

FIG. 4. Spindles of the second nuclear division of the macrospore, showing transverse axis and reduction number of chromosomes of micropylar spindle, and longitudinal axis and increased chromatin mass of the antipodal spindle. Z$\frac{1}{12}$ L4. Iron alum.

FIG. 5. Spindles of the second division. Z$\frac{1}{12}$ L4. Iron alum.

FIG. 6. The four nuclei of the second division completed. Z$\frac{1}{12}$ L4.

FIGS. 7–7*a*. The four nuclei, showing persistence of spindle fibers and shifting of nuclei. B & L$\frac{1}{12}$ R4.

FIG. 8. The four nuclei much shifted. B & L$\frac{1}{12}$ R4.

FIG. 9. An unusual antipodal spindle, showing several poles and continuous looping of chromatin band. Z$\frac{1}{12}$ Z18. Iron alum.

FIG. 10. The micropylar spindle of the same sac, showing several poles and two masses of continuous looped chromatin band. Z$\frac{1}{12}$ Z18. Iron alum.

FIG. 11. An antipodal spindle with numerous poles. Z$\frac{1}{12}$ Z18. Iron alum erythrosin.

FIG. 12. Micropylar spindle of same sac, showing several poles and a continuous looping of the chromatin band. Z$\frac{1}{12}$ Z18. Iron alum erythrosin.

FIG. 13. Spindles of the third nuclear division, showing transverse synergid spindle, longitudinal polar nuclei spindles, and an antipodal spindle. $Z\frac{1}{12}$ L4.

FIG. 14. Same stage, with radiations more evident, equatorial thickening, and a very evident antipodal spindle. $Z\frac{1}{12}$ L4.

FIG. 15. Same stage further advanced. $Z\frac{1}{12}$ L4.

FIG. 16. Completed eight-celled stage. $R\frac{1}{7}$ R4.

FIG. 17. Embryo sac, showing wall at antipodal and micropylar ends, the latter cutting off the synergids, the former one antipodal cell, and the definitive nucleus dividing. B & $L\frac{1}{12}$ R4.

FIG. 18. *L. tigrinum;* polar nuclei fusing. B & $L\frac{1}{12}$ R4.

FIG. 19. *L. tigrinum;* pollen tube with the second male nucleus disintegrating and the tip of the tube ruptured; fusing sex nuclei, the male uppermost; fusing polar nuclei, but the small part of the upper one showing. B & $L\frac{1}{12}$ R4.

FIG. 20. *L. tigrinum;* pollen tube bent sharply towards oosphere, with disorganized tip, the nucleus near its tip being that of persistent synergid; fusing sex nuclei, the male on the left. B & $L\frac{1}{12}$ R4.

FIG. 21. Fusing sex nuclei, with paired centrosomes. B & $L\frac{1}{12}$ R4.

FIG. 22. *L. tigrinum;* fusion of sex nuclei about completed; persistent synergid nucleus above. B & $L\frac{1}{12}$ R4.

FIG. 23. Oospore with broad basal attachment, and the fusion nucleus in apical position; nucleus of persistent synergid still visible. B & $L\frac{1}{12}$ R4.

FIG. 24. Young embryo, showing first division transverse, second division basal and longitudinal; pollen tube with ruptured tip and a remarkably persistent male nucleus. B & $L\frac{1}{12}$ R4.

FIG. 25. Same, but second division transverse. B & $L\frac{1}{12}$ R4.

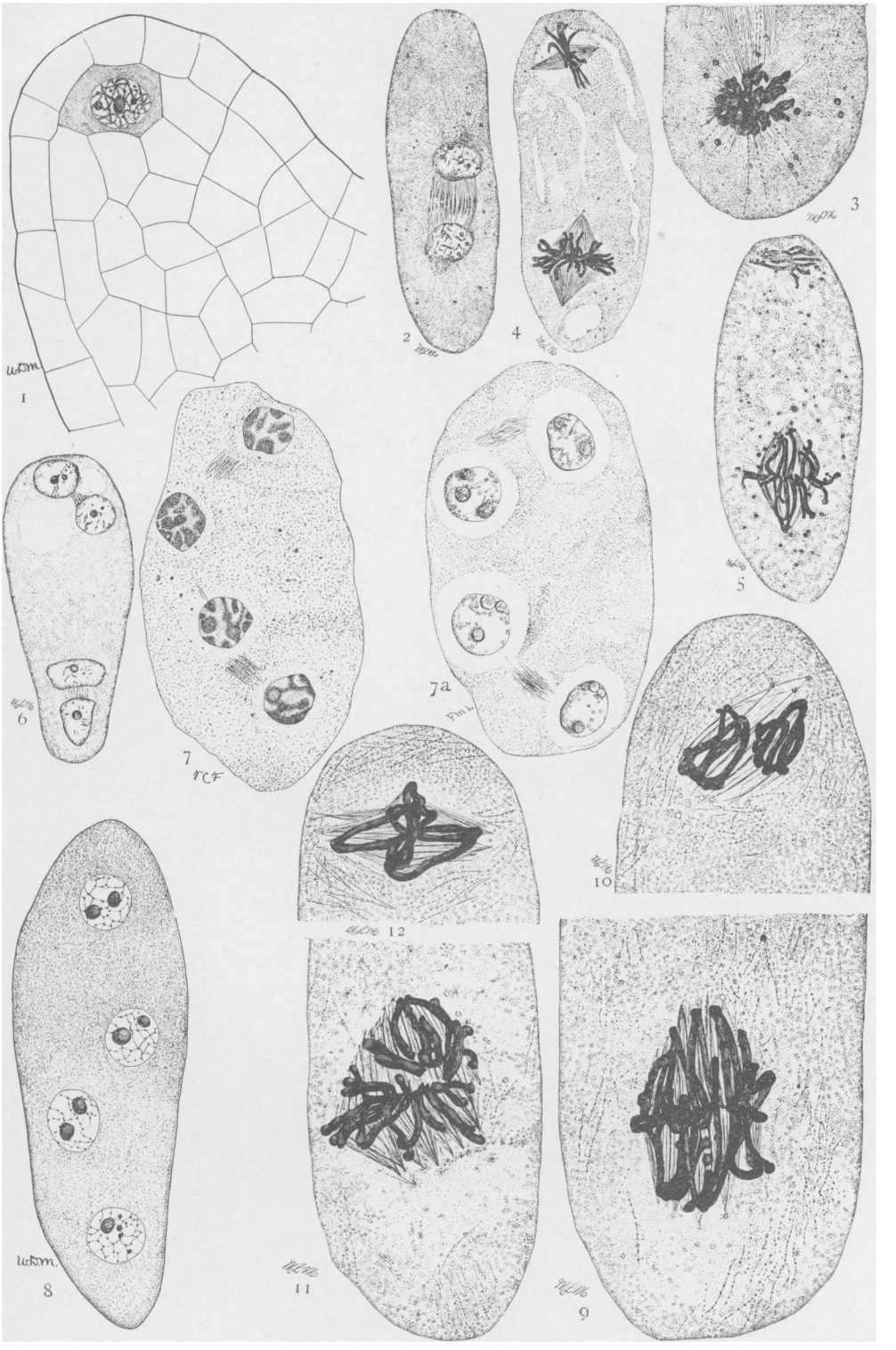
FIGS. 26–30. Young embryos, showing various phases of division and the broadened basal region. B & $L\frac{1}{12}$ R4.

FIGS. 31–32. More advanced embryos. B & $L\frac{1}{12}$ R4.

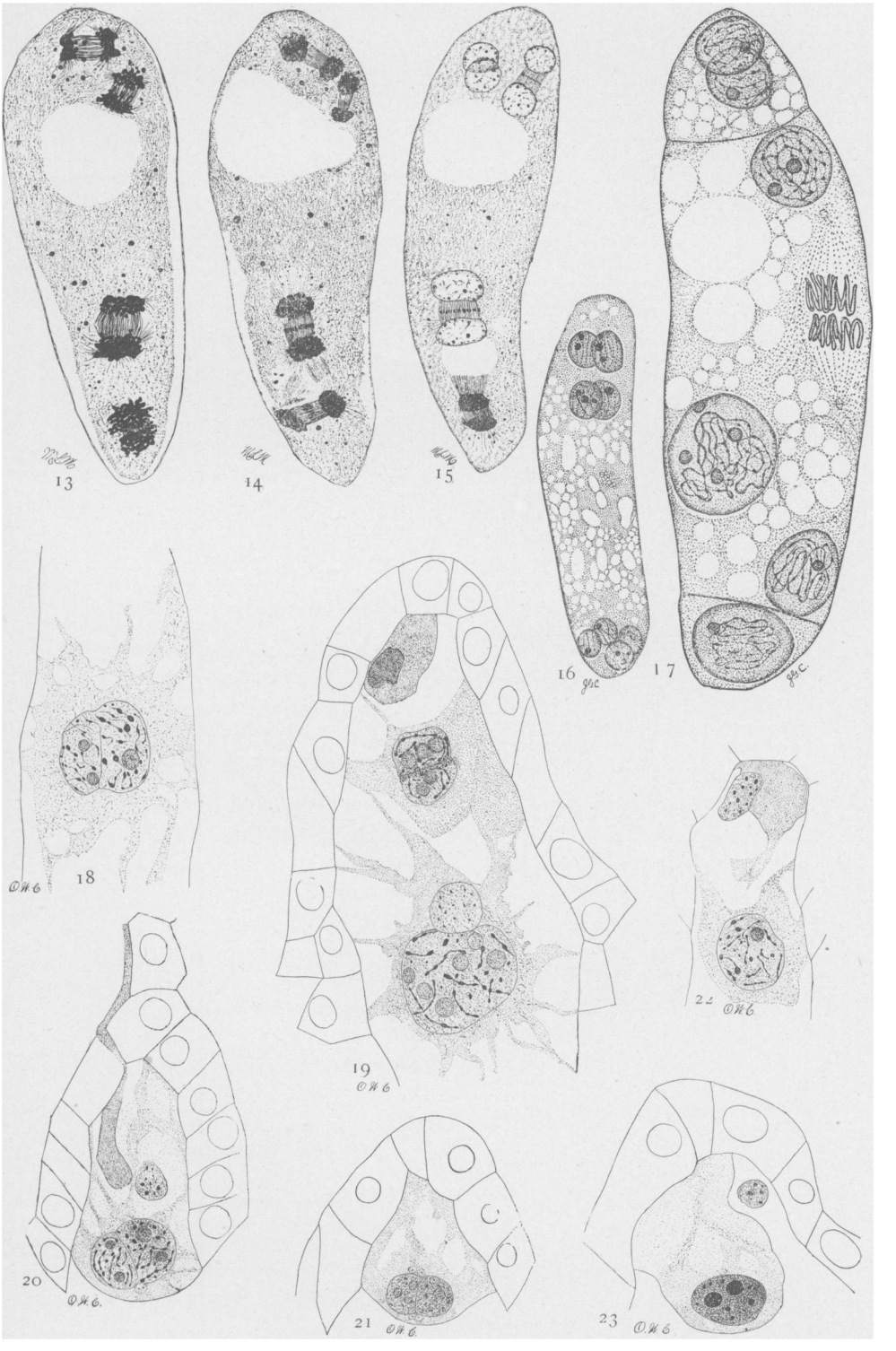
FIG. 33. Advanced embryo, showing development of the suspensor region which is separated by a narrow neck from the embryo proper. $R\frac{1}{7}$ R4.

FIG. 34. *L. tigrinum.* First division of definitive nucleus, showing remarkably prominent radiations about the daughter nuclei. B & $L\frac{1}{12}$ R4. Safranin and gentian violet.

FIG. 35. Embryo sac, showing free endosperm nuclei, the caecum-like antipodal end of the sac containing the three disorganizing antipodal nuclei, and a three-celled embryo. $R\frac{1}{7}$ R4.



COULTER on LILIUM.



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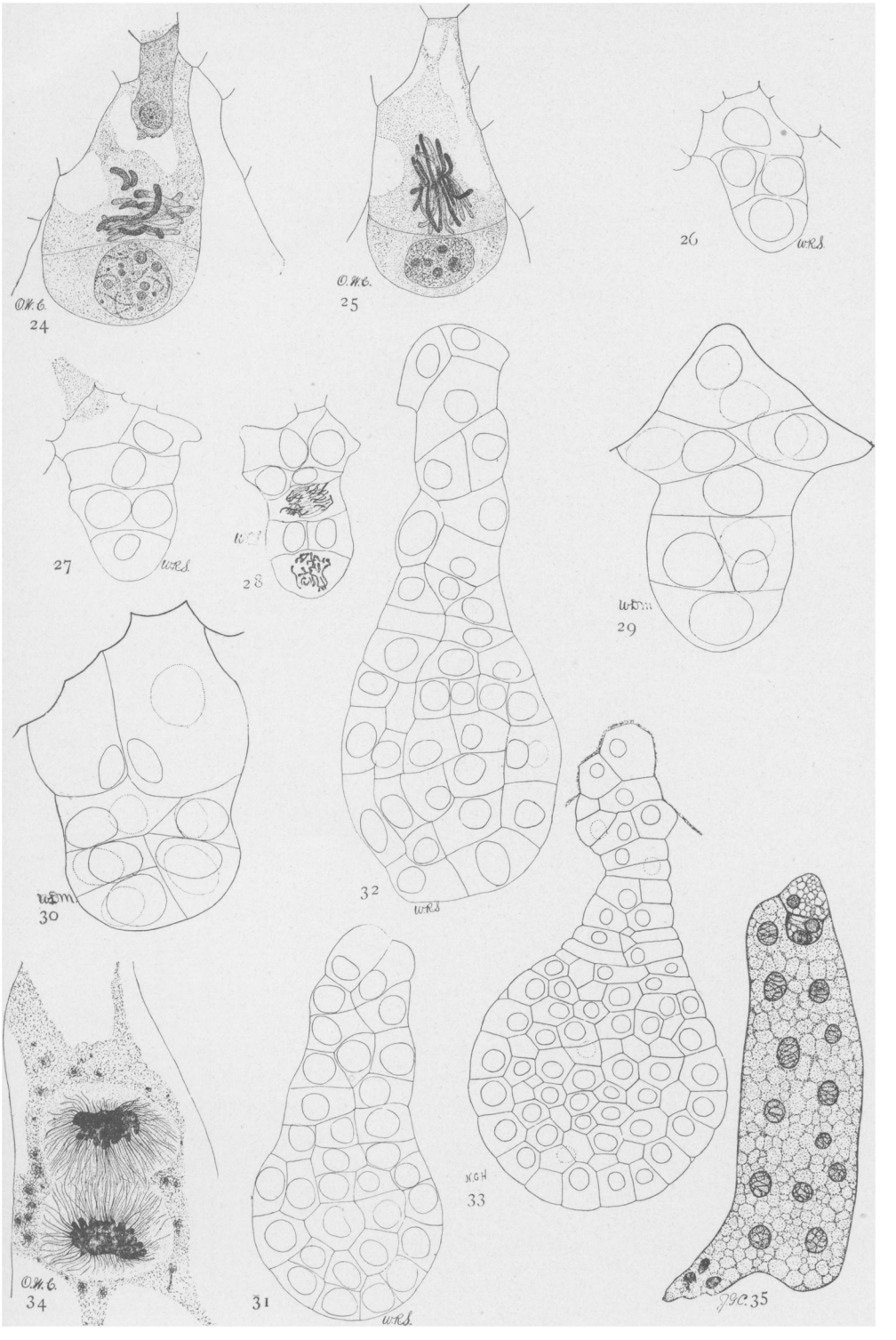

COULTER on LILIUM.